Evaluation of Erythrocyte Levels of Long-chain Omega-3 Fatty Acids as a Biomarker for Breast Cancer

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Abstract

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The metabolic syndrome (MetS) increases the risk of breast cancer in postmenopausal women. Importantly, there are few reliable biomarkers for predicting risk for breast cancer. Because long-chain omega-3 fatty acids found in cold water fish may be protective against breast cancer and the MetS, we propose to evaluate the potential of erythrocyte levels of omega-3 fatty acids to serve as a blood biomarker for breast cancer risk. The omega-3 index measures the percentage of the long-chained omega-3 fatty acids, eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3), to total erythrocyte fatty acids(1). The fatty acid composition of the erythrocytes was analyzed in relationship to obesity (BMI) and several other aspects of breast cancer risk in 25 women. In our cohort, the omega-3 index for women at-risk for breast cancer was 3.89% $\pm$ 1.28 which is substantially lower compared with the 8% recommended omega-3 index by Harris for decreasing MetS risk. The omega-3 Index could be a useful biomarker for breast cancer, especially if used in conjunction with other risk factors, for predicting breast cancer risk in women. 1) Harris WS, Am J Clin Nutr 2008; 1999S. Funding from the Fisheries Scholarship Fund and the Food Innovation Center (LDY).

Introduction

There are currently no reliable blood biomarkers to assess breast cancer risk. There is the potential that long chain omega-3 fatty acids could be used as a biomarker to assess breast cancer risk. Eicosapentanoic acid (EPA; 20:5n3) and Docosahexaenoic acid (DHA; 22:6n3) are omega-3 polyunsaturated fatty acids (PUFA). Fish oils are a rich source of omega-3 fatty acids. Previous research has shown that diets supplemented with EPA and DHA suppressed tumor growth and metastasis of transplanted human mammary cancer in nude mice (1,2). Consumption of EPA and DHA has shown reduction in the risk of coronary heart disease by lowering triglyceride levels and blood pressure (3), and potential anti-cancer effects (4).

Studies have shown that long-chain omega-3 fatty acids have anti-inflammatory effects and may suppress tumor growth in mouse models for breast cancer. Omega-6 fatty acids have the opposite effect in mice, having an inflammatory effect and stimulatory effect on tumor cell growth (1). The ratio of these omega-3 fatty acids to omega-6 fatty acids in the body may be important in mammary carcinogenesis. Studies have shown an inverse relationship between this ratio and breast cancer (5).

Erythrocytes are a strong indicator of long-term omega-3 dietary intake. This is because omega-3 fatty acids are incorporated into the phospholipids of the cell membranes during both reticulocyte maturation and through plasma exchange making erythrocytes an accurate indicator of dietary fatty acid intake. The Omega-3 Index is the sum of EPA+DHA in erythrocyte membranes as a percentage of total erythrocyte fatty acids. When the omega-3 index was compared with other coronary heart disease risk factors such as cholesterol, triacylglycerols LDL, and HDL, C reactive proteins, and homocysteine only the omega-3 index and C reactive proteins had a statistically significant relation with risk for sudden cardiac death (6). However, there have not been any studies looking at the omega-3 index and breast cancer risk. The proposed cutoffs of <4% (low omega-3 index), 4-8% (intermediate omega-3 index), >8% (high omega-3 index) were determined as cardioprotective target concentrations based on a review of the literature of what omega-3 index values were associated with the lowest risk for coronary heart disease (6).

Breast cancer screening and early detection are important because they can increase survival rates and decrease the seriousness of the treatment needed. The Gail Model is used as a breast cancer risk assessment score. It takes into account a women's age, age at first period, age at the time of birth of her first child, number of first degree relatives wtih breast cancer, number of past breast biopsies, number of breast biopsies showing atypical hyperplasia, and race (7). The Gail Model was created based on analysis of Caucasian women in the Breast Cancer Detection Demonstration Project. It has been updated to assess the risk of African American, Asian, and Pacific Islander women. It calculates both a 5 year and lifetime risk assessment score. A score greater than or equal to 1.66% is associated with an increased risk for breast cancer. The tool measures absolute risk which determines the probability of developing breast cancer in a defined age interval. The Gail Model has been validated for Causian, Asaian, and Pacific Islanders but may underestimate the risk of African Americans.

Methods

Packed erythrocyte fractions were obtained from whole blood samples and stored at -80°C. These samples were obtained as baseline measurements from 25 women at high risk for breast cancer who were participating in a clinical trial with an omega-3 fatty acid intervention at the Stefanie Spielman Comprehensive Breast Center. Fatty acids were extracted and methylated from red blood cells using boron trifluoride and the samples were heated for 10 minutes at 100°C (8). Samples were centrifuged at room temperature at 3630 x g for 3 minutes and the hexane layer, which contains the lipid-soluble components including fatty acid methyl was obtained, and inserted in a vial for analysis by gas chromatography.

Analysis of fatty acid methyl esters was completed by gas chromatography using a 30-m Omegawax™320 fused silica capillary column (Supelco, Bellefonte, PA). Oven temperature started at 175˚C and increased at a rate of 3˚ C/min until reaching 220˚C. Flow rate of the carrier gas helium was 30 mL/min. Retention times of samples were compared to standards for fatty acid methyl esters (Matreya, LLC, Pleasant Gap, PA, Supelco, Bellefonte, PA and Nu-Check Prep Inc, Elysian, MN). Fatty acids are reported as total identified fatty acids in erythrocyte samples (9).

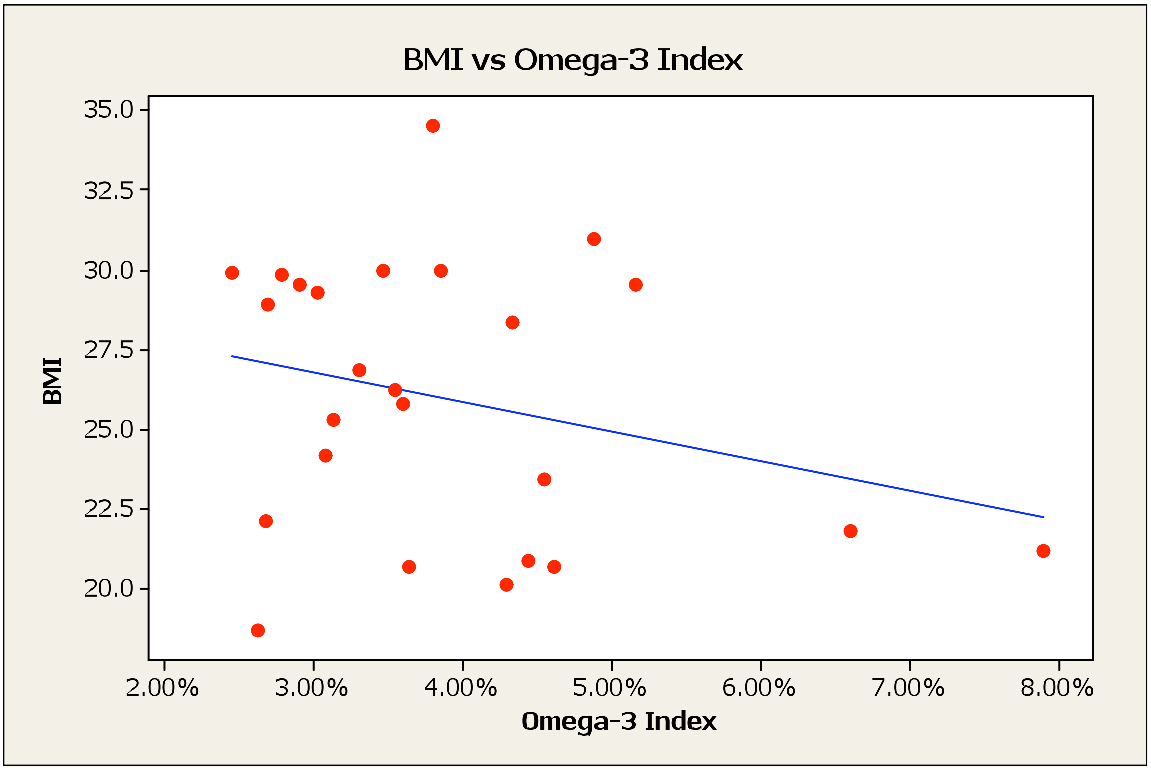
Results

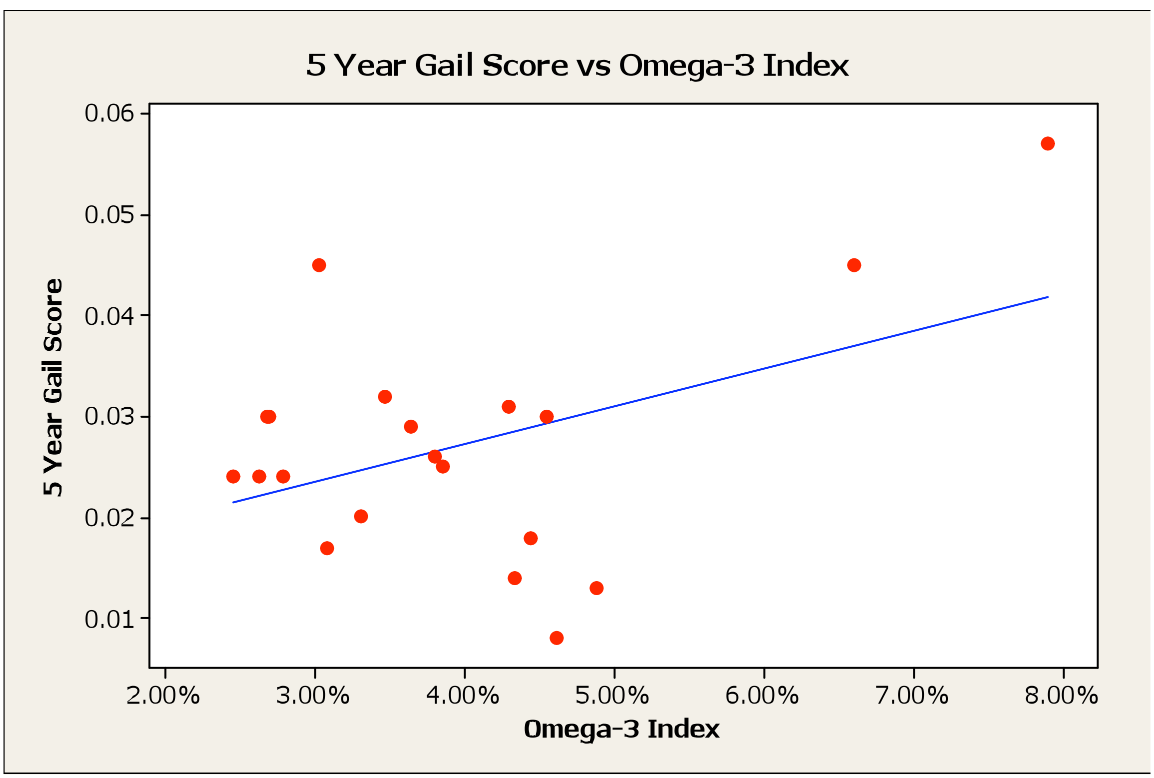
All subjects had an Omega-3 index that was < 8%. Fourteen subjects had an Omega-3 Index <4% and 9 subjects had an Omega-3 Index between 4-8%. The average Omega-3 Index for our cohort of 25 women was 3.89% + 1.28%. In this cohort of women with an omega-3 index less than 4% compared with those with an omega-3 index greater than 4%, there appeared to be no significant relationship with some predictors of the metabolic syndrome (Table 1: e.g., age, waist to hip ratio, and waist circumference or BMI.) All of the subjects were classified as high risk for breast cancer (Table 2). Risk factors for breast cancer included family history, high risk histology, personal history of breast cancer, and their Gail Score. The average percentage of erythrocyte membrane fatty acids was calculated for the women with an omega-3 index < 4% and for the women with an omega-3 index between 4 and 8%. There was a significant difference between their DHA, EPA, total omega-3 fatty acids, and total omega-6 fatty acids. There was not a significant difference between their total saturated fatty acid content or their total monounsaturated fatty acid content (Table 3). There was not a strong correlation between BMI and omega-3 index (Graph 1). A higher Omega-3 Index correlated with a higher Gail Score for both the 5 year Gail Score and the Lifetime Gail Score (Graph 2 and Graph 3).

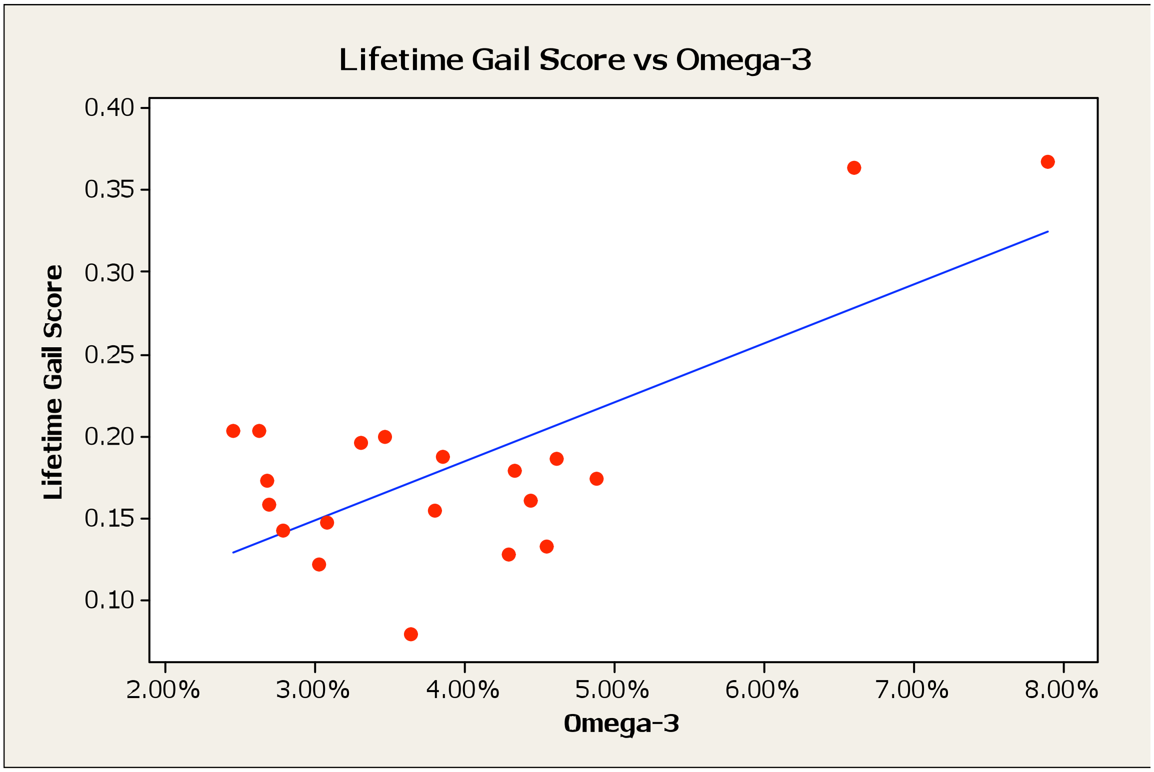
Table 1: Demographics of the Subjects				
		Omega-3 index < 4%		
Characteristic	**Overall (n = 25)**	**Yes (n = 16)**	**No (n = 9)**	**p value**
Age (y)[1]	51.96 + 13.74	51.19+ 9.05	53.33+ 20.24	0.717
Body mass index[1]	25.95 + 4.31	26.99 + 4.09	24.10 + 4.29	0.109
Waist to Hip Ratio[1]	0.82 + 0.06	.82 + .07	.82 + .05	1
Waist (cm)[1]	84.93 + 11.63	86.39 + 11.60	82.33 + 11.91	0.414
1. Mean + Standard Deviation				

Table 2. Classification of Breast Cancer Risk				
Risk Factor	***n* subjects**			
Family history	20			
High risk histology	4			
Personal hx breast CA	1			
Gail score	**Total scored**	**>1.67**	**<1.67**	**Not applicable**
	20	17	3	5

Table 3. Average Percentage of Erythrocyte Membrane Fatty Acids				
		Omega-3 Index < 4%		
		Yes (n=16)	**No (n=9)**	**p**
EPA[1]	0.51 ± 0.23	0.40 ± 0.12	0.72 ± 0.25	<.001
DHA[1]	3.37 ± 1.09	2.76 ± 0.45	4.48 ± 1.02	<.001
Omega-3 Index[1]	3.89 ± 1.28	3.16 ± .45	5.19 ± 1.24	<.001
Total Omega-3 Fatty Acids[1]	6.52 ± 1.30	5.88 ± 0.58	7.67 ± 1.46	<.001
Total Omega-6 Fatty Acids[1]	35.35 ± 2.25	36.03 ± 2.37	34.13 ± 1.42	0.040
Total Saturated Fatty Acids[1]	44.57 ± 1.88	44.53 ± 2.03	44.64 ± 1.70	0.892
Total Monounsaturated Fatty Acids[1]	13.56 ± 0.88	13.56 ± 0.78	13.56 ± 1.09	1
1, Mean ± Standard Deviation				


BMI vs Omega-3 Index
BMI
35.0
32.5
30.0
27.5
25.0
22.5
20.0
2.00%
3.00%
4.00%
5.00%
6.00%
7.00%
8.00%
Omega-3 Index


5 Year Gail Score vs Omega-3 Index
5 Year Gail Score
0.06
0.05
0.04
0.03
0.02
0.01
2.00%
3.00%
4.00%
5.00%
6.00%
7.00%
8.00%
Omega-3 Index



Discussion

Our overall hypothesis was that a higher omega-3 index would correlate with a lower risk for breast cancer. However, we found that a higher Omega-3 Index correlated with a higher Gail Score for both the 5 year Gail Score and the Lifetime Gail Score. These findings are in opposition to our hypothesis. This could be due to the small sample size of our study and the homogeneity of the subjects. All the women were from central Ohio, classified as high risk for breast cancer, and were not allowed to be taking omega-3 fish oil supplements for 120 days before the study. In addition, there appears to be two outliers. When this data is excluded, the correlation does not indicate that a higher Omega-3 Index correlated with a higher Gail Score for both the 5 year Gail Score and the Lifetime Gail Score.

All of the women were at a high risk for breast cancer and none of the women had an omega-3 index greater than 8%. This supports our hypothesis that those with a high risk for breast cancer will have a lower omega-3 index score.

Limitations and Future Directions

Limitations in this study include the small, homogenous sample population. A larger, broader population of subjects from throughout the country would be beneficial to analyze. It would also be beneficial to look at women who have not already been classified as at high risk for breast cancer.

Future directions of the study should evaluate the dietary intake of fish oils using food frequency questionnaires to determine if foods are contributing to the polyunsaturated fatty acid profile, saturated fatty acid profile, and monounsaturated fatty acid profile. Further analysis of data will evaluate the relationship of individual fatty acids, e.g., DHA and EPA, with Gail scores.

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